Immunocytological Comparison of Native and Wound Periderm Maturation in Potato Tuber

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ABSTRACT

The maturation of potato (*Solanum tuberosum* L.) tuber native periderm and wound periderm, which develops to replace the native periderm when it is damaged, are agriculturally important processes that are poorly understood. While both types of periderm form from a phellogen layer that serves as a lateral meristem, there has been little research done on comparing the biochemical processes and steps involved in the maturation of the two types of periderm. Here, we use immunological techniques to compare some of the cell wall changes during wound and native periderm maturation. Consistent with our recent work on native periderm, we demonstrate that toluidine blue O is also useful for distinguishing between suberized and non-suberized cells in wound periderm. More importantly, we use the immunological probes JIM5 and JIM7 to show that there is no increase in either un-esterified or esterified homogalacturonan pectin epitopes in phellogen walls accompanying wound periderm maturation. In contrast, as we previously described, native periderm maturation and resistance to excoriation (skinning) is accompanied by an increase in relatively un-esterified and esterified homogalacturonan pectin epitopes in the walls of phellogen cells. These results demonstrate that the biochemical processes responsible for maturation and resistance to excoriation differ between native and wound periderm. This dissimilarity between wound and native periderm maturation demonstrates the potential limitations in applying the wound periderm model to research on native periderm.

RESUMEN

La maduración del peridermo nativo del tubérculo de papa (*Solanum tuberosum* L.) y del peridermo regenerado, el cual desarrolla para reemplazar al peridermo nativo cuando este sufre daños, es un proceso agronómicamente importante que es insuficientemente comprendido. Mientras ambos tipos de peridermo se forman a partir de una capa de felógeno que sirve como meristemo lateral, muy poca investigación se ha hecho comparando los procesos bioquímicos involucrados en la maduración de los dos tipos de peridermo. Aquí, usamos técnicas inmunológicas para comparar algunos de los cambios en la pared celular durante la maduración del peridermo nativo y del regenerado. Consistentemente con nuestro trabajo reciente sobre peridermo nativo, demostramos que el azul de toluidino O es también útil para distinguir las células suberizadas y no suberizadas en el peridermo regenerado. Más importante aún, hemos utilizado los probadores inmunológicos JIM5 y JIM7 para demostrar que no hay incremento de los epitopes de la pectina homogalacturona esterificada y no esterificada en las paredes de las células del felógeno. Estos resultados demuestran que los procesos bioquímicos responsables para la maduración y resistencia a la excoriación son diferentes para el peridermo nativo y para el peridermo regenerado. Esta disimilaridad entre la maduración del peridermo nativo y la del regenerado demuestra las limitaciones potenciales que existen para aplicar el modelo del peridermo regenerado para investigar sobre el peridermo nativo.

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ABBREVIATIONS: BSA, bovine serum albumin; HG, homogalacturonan; PBS, phosphate-buffered saline; TBO, toluidine blue O.

INTRODUCTION

The potato periderm forms the primary protective barrier at the surface of the tuber. When the native periderm is damaged, a wound periderm forms internal to the damaged tissue. Both types of periderm form from periclinal divisions of a cork cambium or phellogen layer, which serves as a lateral meristem (Artschwager 1924; Peterson and Barker 1979). In native tuber periderm, the phellogen layer differentiates from the hypodermis early during development of the tuber (Artschwager 1924; Peterson and Barker 1979). Both the phelloderm and the phellem are derived from periclinal divisions of the phellogen, the phellem forming the protective suberized skin of the tuber (Lulai 2001). Immature native periderm is susceptible to skinning injury (excoriation), which leads to potato loses during storage. As the periderm matures, it becomes resistant to excoriation.

In the case of wound periderm, the phellogen layer develops amid parenchyma cells under the wound surface and becomes detectable after the closing layer begins to form (Thomson et al. 1995; Lulai 2001). As with native periderm, both wound phelloderm and phellem cell layers develop from the phellogen. The resulting wound periderm is located between the closing layer and the remainder of the tuber. Generally, the cellular outlines of wound periderm appear less organized than that of native periderm. As is the case with native periderm, an immature wound periderm is susceptible to excoriation. Excoriation of the wound periderm formed after a potato tuber is cut for seed can lead to infection of the seedpieces with decay organisms, and excoriation of the wound periderm formed before tubers are put into storage can lead to losses from dehydration, disease, and various defects.

An immature periderm has a phellogen layer made up of cells with thin radial walls that fracture easily, allowing the phellem to excoriate or scuff-off in both native (Lulai and Freeman 2001) and wound periderm (Sabba and Lulai 2002). These walls strengthen and thicken during maturation, resulting in resistance to excoriation in both types of periderm (Lulai 2002).

Pectins are one of the primary constituents of the plant cell wall, functioning as an agent in hydration and cell-to-cell adhesion. One of the most common types of pectin is HG, which is a polymer of D-galacturonic acid with varying degrees of methyl esterification (Thakur et al. 1997). We have previously shown that increases in phellogen cell wall pectins accompany the thickening of phellogen radial walls upon maturation in native periderm (Sabba et al. 2002). While there are similarities between native and wound periderm, we have shown previously that there are histological differences in the staining patterns of cell wall polymers involved in the maturation of native and wound periderm (Sabba and Lulai 2002). In this study, we utilized immunological probes to analyze the deposition of cell wall pectin polymers during the maturation of wound periderm and compared these results with our recent findings for native periderm. This is the first report that we know of that directly compares the immunological properties of tuber pectin polymers during maturation of native periderm with wound periderm.

MATERIALS AND METHODS

Preparation of Periderm Sample Material

Tubers (cv Russet Burbank) harvested early, after the growing season, were susceptible to skinning and were therefore considered to be immature. This was confirmed by mechanically testing skinning susceptibility as described previously (Lulai and Orr 1993). Tubers from this harvest that were stored for at least 4 wk under 96% relative humidity at 21 C in the dark were resistant to skinning and were therefore considered mature. Tubers harvested late, after the growing season, were also resistant to skinning and were therefore considered mature.

Tubers were reproducibly wounded using a surgically sharp industrial steel blade to cleanly shave away a 0.75-mm-thick slice of tissue from either the natural tuber surface or the rough cut surface of a tuber that was first cut in half (stem to bud end) with a conventional blade. This technique produced surfaces that were more sharply cut than those obtained with a conventional knife and consequently wound-healed with greater uniformity. A wound periderm was allowed to develop under optimal conditions (Morris et al. 1989), at 21 C and 96% relative humidity in the dark for the desired time period (either 7 d for immature periderm or 18 to 29 d for mature periderm). Wound periderm that was 1 wk old was susceptible to excoriation and was therefore considered to be immature. Wound periderm that was more than 2 wk old was resistant to excoriation and was therefore considered to be mature.

Tissue blocks, including periderm (either native or wound), were cut from the tubers and fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.3). Fixed periderm blocks were washed in phosphate buffer (pH 7.3), hand sectioned

with a razor blade and dehydrated through a graded ethanol series (25%-100%). Dehydrated samples were embedded in L.R. White resin (Polysciences Inc., Warrington, PA, USA) over a period of one week and polymerized in BEEM capsules (Ted Pella Inc., Redding, CA, USA) according to manufacturer's instructions.

Immunolabeling and Silver Enhancement

Periderm samples embedded in L.R. White resin were serial-sectioned with an ultramicrotome (purple reflectance, 150-200 nm) and placed onto silane-treated glass slides (Sigma Chemical Co., St. Louis, MO, USA). One set of sections was stained with 0.1% TBO (Sigma Chemical Co., St. Louis, MO, USA) to distinguish and identify all cell walls present in the sections. The other sections were blocked with 2% BSA in PBS for 1 h, then incubated with a monoclonal antibody diluted with 1% BSA in PBS for either 3 hrs at room temperature, or overnight at 4 C. The antibodies used were either JIM5 diluted 1:200 (binds to relatively un-esterified pectin), or JIM7 diluted 1:100 (binds to relatively esterified pectin) (Knox et al. 1990). JIM5 and JIM7 antibodies were gifts of J. P. Knox, University of Leeds, Great Britain. One set of sections were incubated in 1% BSA in PBS instead of diluted antibody, to serve as a negative control. Samples were washed 4x in 1% BSA in PBS and incubated in goat anti-rat IgG coupled to 5 nm colloidal gold (EY laboratories, San Mateo, CA) for 1 h. Samples were washed 4x with 1% BSA in PBS and 5x with water. Immunolabeling was silver-enhanced with Intense M Silver Enhancement kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's instructions and digital images were taken with a Zeiss AxioCam camera at 400x magnification (Carl Zeiss Inc., Thornwood, NY, USA). All experiments were repeated at least three times with different samples. Sections that were not incubated in a primary antibody did not exhibit labeling after silver enhancement.

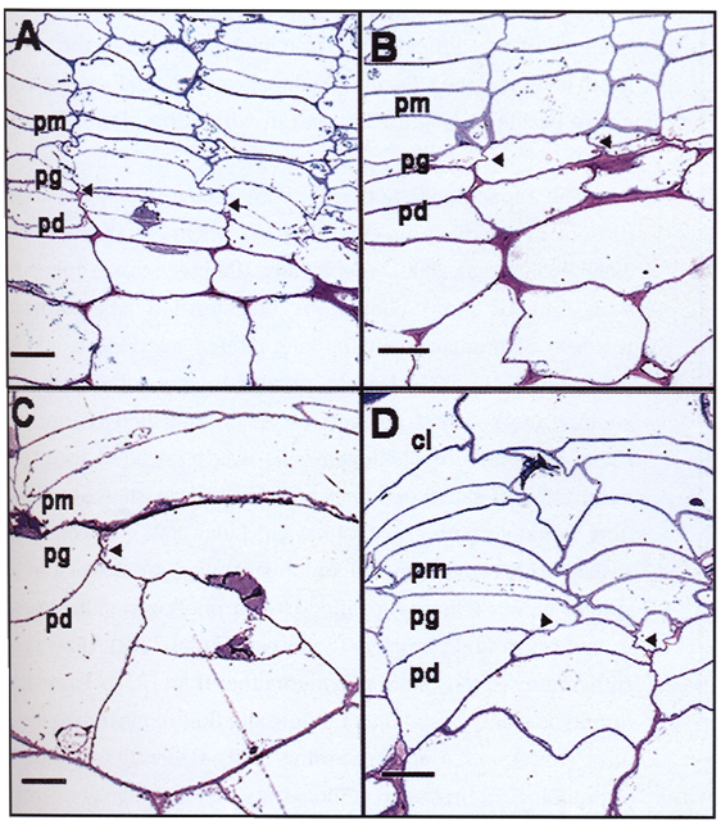

FIGURE 1.
TBO staining of (A) immature native, (B) mature native, (C) immature wound and (D) mature wound periderm. Phellogen radial walls are indicated by arrowheads. Bar = 30 µm, cl = closing layer, pm = phellem, pg = phellogen, pd = phelloderm. The closing layer is visible only in Figure 1D.

RESULTS

Toluidine Blue O Staining

TBO stained the unsuberized phellogen and phelloderm walls metachromatically (violet), but stained the suberized walls of phellem cells orthochromatically (blue), in both native (Figure 1A, B) and wound periderm (Figure 1C, D). The cell walls of the closing layer, i.e., parenchymal cells at the wound surface that rapidly suberize prior to the formation of a wound periderm, also stained orthochromatically (Figure 1D).

JIM5 Immunolabeling

JIM5 is a monoclonal antibody that preferentially binds to relatively unesterified pectin (Knox et al. 1990). JIM5 labeled the walls of phelloderm cells in both immature and mature native periderm, but labeled the walls of phellem cells poorly (Figure 2A,B). In immature native periderm, the walls of phellogen cells labeled weakly with JIM5 (Figure 2A). However, in mature native periderm, these walls labeled strongly with JIM5 (Figure 2B).

Labeling for relatively un-esterified pectin in wound periderm was weak compared to labeling of native periderm (Figure 2). However, the labeling pattern was similar. The walls of phelloderm cells were

labeled, while those of the phellem cells labeled very poorly in both immature and mature wound periderm (Figures 2C, D). In addition, the radial walls of phellogen cells also labeled weakly with JIM5 in both immature and mature wound periderm (Figures 2C, D).

JIM7 Immunolabeling

JIM7 is a monoclonal antibody that binds to relatively esterified pectin (Knox et al. 1990). JIM7 labeled phellem and phelloderm cell walls in both immature (Figure 3A) and mature (Figure 3B) native periderm. Labeling of phellogen walls, especially radial walls, was very weak in immature native periderm (Figure 3A). JIM7 labeling of phellogen walls was much more intense in mature periderm (Figure 3B). In both immature and mature wound periderm, JIM7 labeling was very weak (Figures 3C, D). In particular, labeling of phellogen walls was weak in both immature and mature wound periderm (Figures 3C, D).

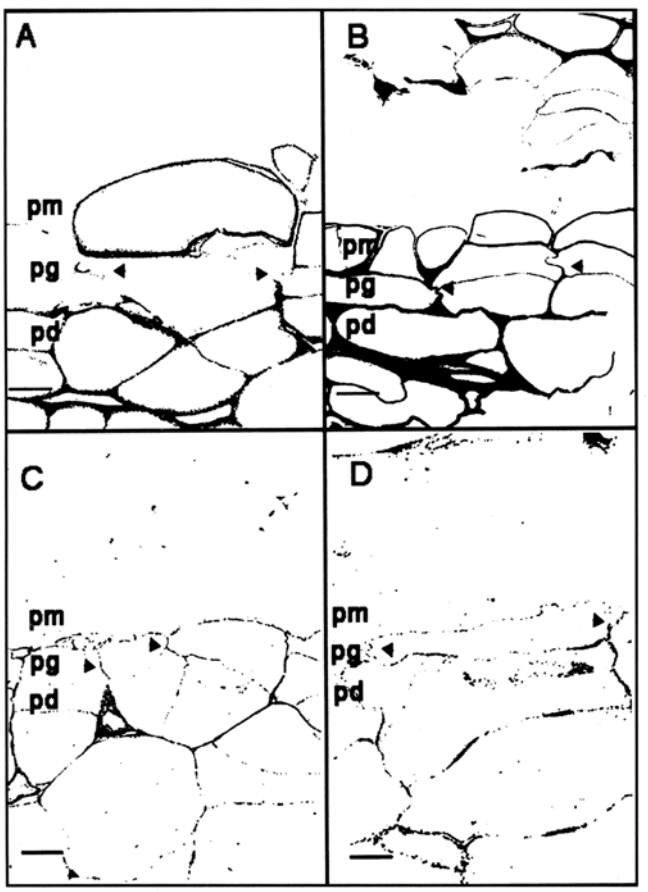

FIGURE 2.
Immunolabeling of (A) immature native, (B) mature native, (C) immature wound and (D) mature wound periderm with JIM5 for an un-esterified homogalacturonan epitope. Note the increase in labeling of phellogen radial walls (indicated by arrowheads) in mature native periderm and the reduced labeling in wound periderm along with the near absence of labeling in wound phellogen cells. Bar = 30 μm, pm = phellem, pg = phellogen, pd = phelloderm.

DISCUSSION

TBO staining represents an excellent means of differentiating between the phellem and the phellogen layers in potato tuber periderm. In both native and wound periderm, suberized walls of the phellem stained orthochromatically (blue) while the non-suberized phellogen and phelloderm stained metachromatically (violet) (Figure 1). In addition, the closing layer, which is located external to the wound periderm, stained orthochromatically (Figure 1D), implying that orthochromatic staining was due to the presence of suberized walls. Other means of differentiating suberized from non-suberized walls involve the use of suberin autofluorescence or suberin specific fluorochromes such as neutral red or berberine (Lulai and Morgan 1992). The simplicity of the TBO method is important because of the difficulty in distinguishing between the phellogen layer and the adjacent phellem layer especially in mature native periderm (Figure 1B) and in wound periderm (Figure 1C, D).

The monoclonal antibody JIM5 recognizes an epitope present in relatively un-esterified HG polymers (Knox et al. 1990; Willats et al. 2000). Specifically, JIM5 recognizes epitopes with four or more contiguous un-esterified galacturonan residues with adjacent or flanking methyl-esterified residues (Clausen et al. 2003). Results obtained using ruthenium red staining implied that an increase in un-esterified HG in the radial cell walls of phellogen cells was associated with the maturation of native periderm and onset of resistance to skinning in native periderm (Sabba and Lulai 2002). In contrast, results with ruthenium red staining implied an absence of a similar increase in un-esterified HG for phellogen radial walls in mature wound periderm (Sabba and Lulai 2002). However, ruthenium red may stain polymers other than HG, because its affinity is solely based on a binding site that requires two negative charges 4.2 Å apart (Sterling 1970). Recently we utilized immunological probes to demonstrate that in native periderm, epitopes for both un-esterified and esterified HG increased greatly in phellogen cell walls upon maturation (Sabba et al. 2002). We repeated a portion of these immunolocalization experiments on native periderm to allow direct comparison in

our current investigations on wound periderm. The data on native periderm that we report in this paper are fully consistent with our previous study and confirm that the thin phellogen radial walls of immature native periderm lack epitopes for both un-esterified and methyl-esterified HG, while the thickened phellogen radial walls of mature native periderm are enriched in these HG epitopes (Figure 2). Our data show that the phellogen radial walls of both immature and mature wound periderm are lacking in HG, implying that the maturation of wound periderm is not associated with an increase in HG in phellogen radial walls (Figure 2). These immunospecific results are consistent with the histological data we recently reported (Sabba and Lulai 2002). The distinctly different chemistries used in obtaining similar results in these two separate studies verify unexpected and unpredicted differences between native and wound periderm.

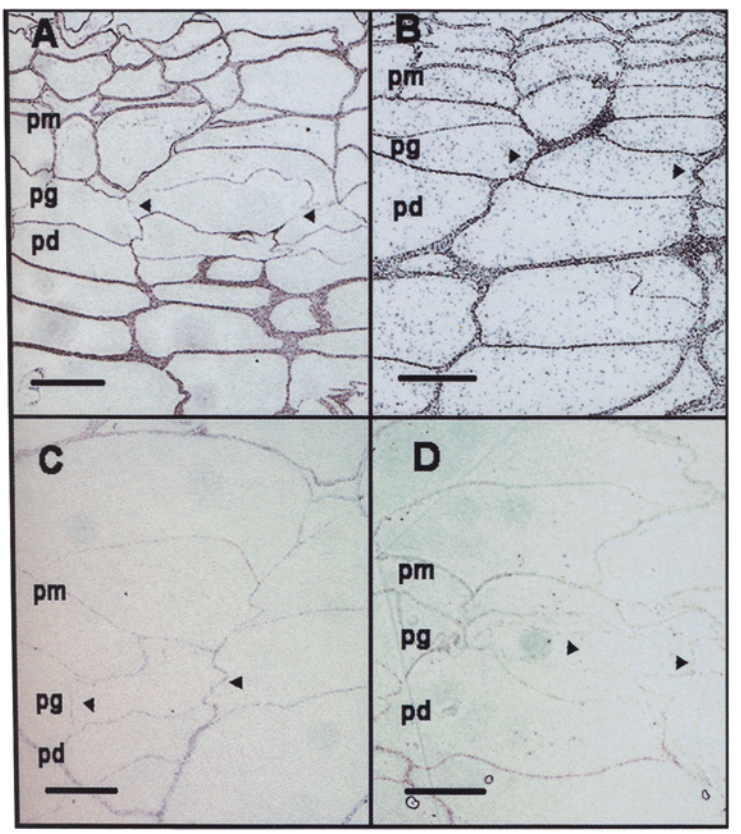

FIGURE 3.
Immunolabeling of (A) immature native, (B) mature native, (C) immature wound and (D) mature wound periderm with JIM7 for a methyl-esterified homogalacturaonan epitope. Note the increase in labeling of phellogen radial walls (indicated by arrowheads) in mature native periderm and the sparse labeling in both immature and mature wound periderm. Bar = 30 µm, pm = phellem, pg = phellogen, pd = phelloderm.

The monoclonal antibody JIM7 recognizes an epitope present in moderately to highly esterified HG (Knox et al. 1990; Willats et al. 2000). Specifically, JIM7 recognizes epitopes with either three or more methyl-esterified galacturonan residues, or alternating methyl-esterified residues, all with adjacent or flanking un-esterified residues (Clausen et al. 2003). JIM7 labeled both phellem and phelloderm cell walls in both immature and mature native periderm (Figure 3A, B). Taken together with the JIM5 labeling pattern, these data indicate that the suberized phellem cell walls do contain HG, but most of it is methyl-esterified. This conclusion is also consistent with results obtained using totally different chemistries, i.e., the histological data showing that ruthenium red stained phellem cell walls, but only after the walls have been chemically de-esterified prior to staining (Sabba and Lulai 2002). Of particular interest, JIM7 labeled phellogen radial walls very weakly in immature native periderm (Figure 3A). In comparison, labeling of phellogen radial walls was intense in mature native periderm (Figure 3B). These results imply that phellogen cell wall thickening resulting from native periderm maturation is accompanied by increases in both un-esterified and esterified HG. JIM7 labeled wound periderm cell walls very weakly (Figure 3C, D). In particular, JIM7 labeled phellogen cells weakly in both immature and mature wound periderm (Figure 3C, D). This result implies that maturation of wound periderm is not associated with an increase in either esterified or un-esterified HG in phellogen radial walls.

The differences between HG labeling of phellogen walls of native and wound periderm imply that different biochemical processes are involved in the strengthening and thickening of phellogen walls accompanying maturation of the two types of periderm. Careful consideration of the differences in maturation and development of resistance to excoriation between the two types of periderm is recommended before making comparisons between native and wound periderm.

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